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Title: The effect of surface treatment of silicone hydrogel contact lenses on the attachment of *Acanthamoeba castellanii* trophozoites

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Focus Night & Day lenses were donated by Ciba Vision, Duluth, USA.

Abstract

Objective: To determine if plasma surface treatment of Focus Night & Day silicone hydrogel contact lenses affects the attachment of *Acanthamoeba*.

Methods: Unworn lotrafilcon A contact lenses with (Focus Night & Day) and with out surface treatment and Acuvue, conventional hydrogel lenses were quartered prior to 90 minutes incubation with *Acanthamoeba castellanii* trophozoites. After incubation and rinsing the trophozoites attached to one surface of each quarter were counted by direct light microscopy. Sixteen replicates were observed for each lens type. Logarithmic transformation of data allowed the use of parametric ANOVA.

Results: No significant difference in attachment was established between the untreated lotrafilcon A lens and the conventional hydrogel ($p<0.001$), however surface treatment of the native Focus Night & Day material produced a significant increase in attachment ($p<0.001$).

Conclusions: Commercially available Focus Night & Day lenses are subjected to a plasma surface treatment to reduce lens hydrophobicity, however this procedure results in enhanced Acanthamoebal attachment. It is possible that the silicone hydrogel lens could be at greater risk of be promoting *Acanthamoeba* infection if exposed to the organism, due to the enhanced attachment characteristic of this material. Eye care professionals should be aware of the enhanced affinity *Acanthamoeba* show for this lens, and accordingly emphasise to patients the significance of appropriate lens hygiene. This is particularly important where lenses are worn in a regime which could increase the chance of exposure to the organism, i.e. 6 night/7 day extended wear or daily wear where lenses will be stored in a lens case, or where lenses are worn when in contact with potentially contaminated water sources, i.e. swimming or shower.

Key word: *Acanthamoeba*, contact lenses, keratitis, eye infection

Ubiquitous free-living protozoa of the genus *Acanthamoeba* cause the ulcerative infection of the cornea, *Acanthamoeba* keratitis (AK). Up to 93% of cases in the developed world have been associated with contact lenses wear.[1] Historically, the rate of infection in the USA has been conservatively estimated to be 0.020 cases/10000 contact lens wearers (CLW).[2] While rates of 0.33 cases/10000 soft CLW have been reported in England and Wales, Europe, Hong Kong and other countries with similar contact lens fitting and hygiene practices.[3] More recently Joslin and colleagues identified 63 cases of AK in the Chicago area between June 2003 and December 2006;[4, 5] these figures being significantly higher than historical rates. This increased incidence prompted the Centers for Disease Control and Prevention (CDC) to undertake a retrospective survey of case reports at ophthalmology centres, the results of which indicated that the increased level of infection was occurring throughout the USA.[6] Other authors in the USA and outwith have noted similar increases in incidence rates.[7-10] The CDC also reported an increased risk of infection with use of the Complete MoisturePlus brand of lens disinfectant (Advanced Medical Optics (now Abbott Medical Optics) Illinois, USA), and despite a global recall of this product, recent work has shown a continuation of higher than historical levels of infection (Gupta, Tu and Joslin IOVS 2009; 50: ARVO E-Abstract 3114).

In addition to ineffective contact lens disinfection systems,[6, 11] other lens associated risk factors for the disease have been cited, i.e. rinsing lenses in tap water,[12, 13] use of home-made saline,[14] and contamination of lens storage cases.[15-17] Such poor hygiene practices allows the organism to attach to the contact lens, a prerequisite for infection as the lens then acts as a mechanical vector transmitting the organism onto the corneal surface, where invasion and subsequent infection can occur.

Acanthamoeba exhibit differing affinities for different contact lens materials.[18-23] Contact lenses composed of etafilcon A had been shown to have the highest attraction for *Acanthamoeba* among conventional hydrogel lenses.[18-20] However the organism has shown a significantly greater affinity for the two brands of first-generation silicone hydrogel (S-H) lenses on the market, Focus Night & Day by Ciba Vision (Southhampton, UK) and Purevision produced by Bausch and Lomb (Kingston-Upon-Thames, UK),[21-24] and also the second generation O_2Optix lens (Ciba Vision; lotrafilcon B; Beattie and Tomlinson IOVS 2006; 47: ARVO E-Abstract 2410). The native materials these lenses are constructed from are intrinsically hydrophobic and are subjected to plasma surface treatment to reduce hydrophobocity. Previous work had suggested that the surface treatment of the Purevision lens may not be responsible for the increased affinity of *Acanthamoeba*, but that the high level of attachment seen may be due to an inherent characteristic of the polymer.[22] The current study determined what effect surface treatment of the Focus Night & Day lens material had on the levels of Acanthamoebal attachment.

Materials and Methods

Contact lenses

Surface treated and non-surface treated Focus Night & Day lenses (lotrafilcon A, 24% H_20), were donated by Ciba Vision. For this experiment attachment was compared with that of the Acuvue, conventional hydrogel lens (etafilcon A, 58% H_20; Johnson & Johnson Vision Care, Jacksonville, USA). The Acuvue lens was chosen as a comparison standard in this, and previous studies by our group, as *Acanthamoeba* have show a greater affinity for this lens compared with other conventional hydrogels.[18-20] All lenses were quartered prior to use, to prevented overlap and folding during preparation for microscopic examination.

Experimental Procedure

The test organism was prepared as described previously.[21] Briefly, *Acanthamoeba castellanii* (CCAP 1501/1A; genotype T4) trophozoites were cultured on non-nutrient agar plates seeded with heat killed *Klebsiella aerogenes* (WPRL CN345). To remove the trophozoites the plates were flooded with Page's amoebal saline (PAS)[25] and a sterile spreader used to gently dislodge the amoebae from the surface of the plates. The PAS containing the trophozoites was centrifuged at 3000 rpm for 10 minutes at room temperature, the supernatant removed, and the *Acanthamoeba* pellet resuspended in 10 ml PAS. Centrifugation and resuspension was repeated a further two times to remove any remaining heat killed bacteria. Saline suspensions were enumerated using a Neubauer haemocytometer and adjusted to 10^5 trophozoite/ml by dilution or centrifugation. Suspensions were dispensed in 1ml volumes into sterile glass bijou bottles for lens incubation.

Unworn lens quarters of each type were incubated individually, on an orbital shaker (80rpm), at 25^{o}C for 90min in 1ml of the saline suspension containing ~10^5 trophozoites. After incubation, quarters were rinsed in PAS for 1min on an orbital shaker (80rpm) then mounted on microscope slides under a coverslip for trophozoite enumeration. The entire surface of each lens quarter was directly scanned via a light microscope at x100 – x200 magnification and all trophozoites attached to one surface counted. Quartering the lenses to allow for microscopic examination resulted in uneven quarters, therefore all quarters where measured to allow counts to be expressed as trophozoites attached/cm^2 of lens. Sixteen replicates were observed for each lens type.

Statistical analysis

Attachment of *Acanthamoeba* to conventional hydrogel and S-H contact lenses has been shown to have an inherently high variability both within and between experimental runs. [18-23, 26] Amelioration of this effect was achieved by comparison of trophozoite attachment to unworn conventional hydrogel lenses among experimental runs and data adjustment via reference to these control values. With the inherent skewness in the variables under consideration, natural logarithmic transformations were necessary before parametric statistical analysis was performed. One factor, balanced analysis of variance was used to analyse the transformed data, with Tukey's pairwise comparison for follow up testing.

Results

The mean, median and standard deviation of trophozoites attached/cm^2 of each lens type are shown in Table 1. Figure 1 shows typical sections of each lens with trophozoites attached.

No significant difference in attachment was found between the untreated lotrafilcon A lens and the conventional hydrogel. However, lens treatment had a significant effect on attachment, with higher numbers of trophozoites found attached to the surface treated lotrafilcon A lenses compared with the native lotrafilcon A material and the conventional hydrogel ($p<0.001$).

Discussion

Acanthamoeba demonstrated a significantly greater affinity for the commercially available, surface treated Focus Night & Day lens compared with the conventional hydrogel. This is consistent with previous work, where higher numbers of trophozoites were found attached to Focus Night & Day lenses and also the Purevision S-H lens compared with a conventional hydrogel.[21-23] However, amoebal attachment to the lotrafilcon A lens which had not been exposed to surface treatment was at a similar level to that found with the conventional hydrogel lens, indicating that surface treatment was responsible for the increased attachment to the commercially available lens.

Contact lenses constructed from S-H material incorporate the structural elements of silicone rubber into a conventional hydrogel, producing a dramatic enhancement of oxygen transmissibility,[27] however, these materials are intrinsically hydrophobic, due to siloxane moieties migrating to the surface.[28, 29] Use of the native material in a lens would lead to decreased wettability and increased lipid interaction compared with a conventional hydrogel.[27] To overcome hydrophobicity, Focus Night & Day and Purevision lenses are subjected to a gas plasma surface treatment procedure. During the plasma treatment of the Focus Night & Day lens reactive precursors fed into the plasma change their structure resulting in deposition as a uniform 25nm thick, dense, high refractive index, organo-nitrogen coating on the lens surface.[30, 31] The Purevision lens is exposed to a plasma oxidation surface treatment, which produces 'glassy silicate islands' on the lens surface, interspersed by unoxidised hydrophobic areas, which are 'bridged' over, due to their relatively small size, by the wettability of the glassy silicate areas.[27]

López-Alemany and colleagues investigated the surface structure of the two brands of first-generation S-H lens and conventional hydrogels, including the Acuvue lens, using scanning electron microscopy, and found a substantial difference between the S-Hs and the conventional hydrogels.[28] The Focus Night & Day lens had a wrinkled surface criss-crossed by straight lines; the wrinkled surface became more ordered toward the border of the posterior surface. The anterior (convex) surface of the Purevision lens had a mosaic-like morphology of wrinkled islets surrounded by smooth rivulets, consistent with the 'glassy silicate islands' theory, but also had circular pores spread randomly over the surface. The posterior (concave) surface did not have the mosaic-like structure but did have many pores. In contrast, the surface of the Acuvue lens appeared slightly wavy, but smooth and homogenous. López-Alemany and colleagues suggested that surface treatment was responsible for the irregular surfaces found with the S-H lenses. It is possible that the increased attachment found with the commercially available, surface treated S-H lenses is due to the irregular surfaces textures produced during treatment.[28]

Initially it was hypothesised that the enhanced attachment of *Acanthamoeba* to the Purevision lens was related to the surface treatment procedure and the areas of hydrophobic material left unoxidised after treatment. Unfortunately, investigation of attachment to untreated Purevision lenses was not possible, as the lenses could not be acquired prior to surface treatment. The effect of surface treatment was however investigated using a substitute lens; the silicone elastomer lens, Silsoft (Bausch & Lomb, Kingston-Upon-Thames, UK) was equally as hydrophobic, required similar surface treatment to increase wettability and was available both before and after treatment.[22] No significant difference in attachment was detected between the Silsoft lens before and after treatment and the Purevision lens.[22] By inference, from the Silsoft lens data, it seemed likely the increased level of attachment found with the Purevision lens was due to an inherent property of the polymer and not surface treatment. The results of the current study suggest that in the case of the Focus Night & Day lens, increased attachment may be due to surface treatment as well as to a property of the polymer. Surface treatment could be a factor in attachment to the Purevision lens, but this could only be confirmed if the lens was evaluated in the untreated state.

Johnson & Johnson have also marketed a S-H lens, composed of galyfilcon A, which has been approved for daily wear under the brand name of Acuvue Advance. This S-H lens is not exposed to a surface treatment procedure, and as such has been referred to as a "second-generation" S-H. The problem of hydrophobicity with this lens is overcome by incorporating an internal wetting agent, called Hydraclear, into the lens material. The Hydraclear molecule is part of the polyvinyl pyrrolidone family (PVP) and readily binds to water and retains moisture.[32] Attachment studies with this lens have shown that *Acanthamoeba* have a significantly lower affinity for this new S-H lens compared with the other two S-H lenses available; attachment is at a level similar to that found with the Acuvue, conventional hydrogel lens.[23] As these Acuvue Advance lenses are not exposed to a plasma treatment, the surface texture may remain smooth, similar to that found with the Acuvue lens,[28] and this may explain the lower levels of amoebal attachment, however scanning electron microscopy studies with the Advance lens would be required to confirm this.

The 'sticky' nature of first-generation S-H lenses for *Acanthamoeba* suggests that these new lenses may present a greater risk of promoting *Acanthamoeba* infection if exposed to the organism than a conventional hydrogel. However, to date few cases of AK associated with S-H lenses have been reported.[33] It should be noted though, that few studies specifically addressed lens type/brand associated with infection, often this information may not be available or lens type is merely reported as soft contact lenses. Of the literature reporting the recent increase in incidence of AK only the study by Ku and colleagues documented infection in relation to S-H lenses; 2 of 12 cases of AK among contact lens users reported use of monthly extended wear S-H lenses (1 Focus Night & Day and 1 Purevision).[10] This lead Ku and colleagues to comment that although not conclusive, the use of S-H which are 'sticky' for *Acanthamoeba* in an extended overnight wear regime may increase the risk of developing AK.[10] The authors also commented that the small number of cases analysed made it difficult to determine the significance of the results attained.

One theory behind the apparent low incidence of AK with first generation S-H lenses despite the high affinity the organism has for the lens, was that the high Dk lenses may have reduced Acanthamoebal binding to corneal epithelium cells, in much the same way that the ultra oxygen permeable S-H lenses reduce corneal hypoxia, resulting in reduced bacterial binding to corneal epithelium cells, this being the first step in initiating microbial keratitis.[34]

Wear regime may also play a role in the low levels of AK found with first-generation S-H lenses, as they have been designed, and have been approved, for thirty days continuous wear before disposal, thus avoiding the need for a lens storage case and lens care solutions, both of which have been cited as risk factors in the development of AK.[11, 13, 15-17, 35] However, any deviation from this continuous wear regime, i.e. 6 night/7 day wear regimes, may negate this benefit.

It is possible that the combination of improved corneal health and continuous wear regimes has resulted in increased patient safety with S-H lenses in relation to *Acanthamoeba* infection. However, with the evolution of newer generation S-H lenses for a variety of wear regimes and their increasing use, i.e. S-Hs prescribed for daily wear spherical fits has increased globally from close to 0% in 2002 to about 20% in 2008,[36] coupled with the current increase in infection rates for AK it would seem timely for a study to look specifically at these factors in relation to AK infection.

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Figure 1 Typical sections of lotrafilcon A lenses (A) with and (B) without surface treatment and (C) etafilcon A lenses after 90 minutes exposure to a suspension containing ~10^5trophozoites/ml (mag. X200).